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Genomic Assessment of Blood-Derived Circulating Tumor DNA in Patients With Colorectal Cancers: Correlation With Tissue Sequencing, Therapeutic Response, and Survival

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Abstract

Purpose—Genomic alterations in blood-derived circulating tumor DNA (ctDNA) from patients with colorectal cancers were correlated with clinical outcomes.

Patients and Methods—Next-generation sequencing of ctDNA (54- to 73-gene panel) was performed in 94 patients with colorectal cancer.

Results—Most patients (96%) had metastatic or recurrent disease at the time of blood draw. The median number of nonsynonymous alterations per patient was three (range, zero to 30). The most frequently aberrant genes were *TP53* (52.1% of patients), *KRAS* (34%), and *APC* (28.7%). Concordance between tissue and blood next-generation sequencing ranged from 63.2% (*APC*) to 85.5% (*BRAF*). Altogether, 74 patients (79%) had one or more nonsynonymous alterations, 69 (73%) had one or more potentially actionable alterations, and 61 (65%) had an alteration actionable by a drug approved by the US Food and Drug Administration (on or off label). Lung metastases correlated with improved survival from diagnosis in univariable analysis. ctDNA of 5% or more from blood tests as well as *EGFR* and *ERBB2* (HER2) nonsynonymous alterations correlated with worse survival (but only *ERBB2* remained significant in multivariable analysis).

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No two patients had identical molecular portfolios. Overall, 65% versus 31% of patients treated with matched ($n = 17$) versus unmatched therapy ($n = 18$) after ctDNA testing achieved stable disease for 6 months or more, partial response, or complete response ($P = .045$); progression-free survival, 6.1 versus 2.3 months ($P = .08$); and survival not reached versus 9.4 months ($P = .146$; all by multivariable analysis).

Conclusion—Patients with colorectal cancer have heterogeneous ctDNA profiles, and most harbor potentially actionable ctDNA alterations. Matched therapy yielded higher rates of stable disease for 6 months or more, partial response, or complete response. ctDNA assessment may have clinical utility and merits further investigation.

INTRODUCTION

Colorectal cancer is one of the most common cancers worldwide. Globally, there were 1.4 million new cases and 693,900 deaths in 2012, with an increase in incidence and mortality rates in developing countries.^{1,2} At diagnosis, approximately 20% of patients have distant metastatic disease.³ For decades, systemic therapy used fluorouracil as the main active agent. Addition of irinotecan and oxaliplatin, as well as the recently developed inhibitors that target VEGF (bevacizumab, aflibercept, and regorafenib) and EGFR (cetuximab and panitumumab), have markedly improved the outcome of patients with metastatic colorectal cancer. However, prognosis remains poor (median progression-free survival [PFS], 10 months; median overall survival [OS], 19 to 28 months; 5-year survival, 10%^{4,5}). Thus there is an unmet need to better understand the clinically relevant biology of colorectal cancer.

The molecular characteristics of colorectal cancer are better understood because of advances in next-generation sequencing (NGS) technology.⁶ Categorizing patients on the basis of their underlying molecular features has been proposed (ie, consensus molecular subtypes),⁷ and several genomic markers are now routinely used in the clinic to guide treatment. Examples of genomically guided US Food and Drug Administration (FDA)–approved therapies include anti-EGFR agents (cetuximab and panitumumab) among patients with wild-type *RAS*^{8–10} and pembrolizumab (anti-programmed cell death protein 1 antibody) for microsatellite instability-high or mismatch repair-deficient colorectal cancer.¹¹ In addition, adding vemurafenib (BRAF inhibitor) to irinotecan and cetuximab demonstrated better clinical outcome among patients with *5RA*^{V600}–mutated colorectal cancer.¹² However, despite the recent progress in targeted therapy approaches, more than 50% of patients do not respond to the aforementioned regimens, and an increased understanding of the molecular underpinnings of the disease is needed. Some of the challenges with tissue-based genomic analyses, which often are performed on archival samples, include the fact that the genomic landscape of cancer can change after therapeutic intervention,¹³ and the sequencing results can be confounded by intra- and intertumoral heterogeneity.^{14,15}

To overcome the challenges of tumor heterogeneity and assess the impact of the clonal evolution that occurs with time and under therapeutic pressure, circulating tumor DNA (ctDNA) is now being actively investigated in diverse cancers.^{16–20} Previous studies using ctDNA analysis of colorectal cancer revealed that *TP53*, *KRAS*, and *APC* were the most commonly altered genes.^{21,22} Here, we provide an in-depth evaluation with clinical

characteristics and therapeutic outcomes of patients with colorectal cancer whose ctDNA was interrogated by clinical-grade NGS.

PATIENTS AND METHODS

Patients

We reviewed the clinicopathologic and outcome data from 94 consecutively tested patients with advanced-stage colorectal cancer at the University of California San Diego Moores Cancer Center; each patient had the ctDNA test performed on their plasma (January 2015 to March 2017). The study followed the guidelines of the University of California, San Diego, Internal Review Board, the Declaration of Helsinki for the PREDICT study (NCT02478931; Profile Related Evidence Determining Individualized Cancer Therapy), and any investigational therapy for which the patients gave consent.

Sequencing, Concordance Rate, Matched Therapy, and Actionability

ctDNA sequencing was performed by Guardant Health and has been previously described (Data Supplement).^{16,23,24} Overall, 76 (81%) of 94 patients had NGS performed on tumor tissue using the FoundationOne assay. The methods have been previously described (Data Supplement).²⁵ Tissue NGS and plasma ctDNA tests were compared by using the kappa statistic (Data Supplement).²⁶ We retrospectively analyzed the treatments given after ctDNA testing and compared the clinical outcomes among patients who received matched and unmatched therapies (Data Supplement).²⁷

Statistical Analysis

Statistical analysis was performed by M.C.S. with SPSS version 24.0 (SPSS, Chicago, IL; Data Supplement).²⁸ The rate of stable disease (SD) for 6 months or more, partial response (PR), or complete response (CR) was compared between patients who received matched or unmatched therapy. SD, PR, and CR were determined according to an assessment by the treating physician. PFS was defined as the time from the beginning of therapy to progression or last follow-up date for patients who did not progress. OS was defined as the time from diagnosis until death or last follow-up date for patients still alive. PFS and OS were analyzed by using the Kaplan-Meier method²⁸ and the log-rank test (univariable analysis); a Cox regression model (multivariable analysis) was used to compare variables. Patients still progression free (for PFS) or alive (for OS) at last follow-up were censored on that date.

RESULTS

Patient Characteristics

We evaluated 94 patients with colorectal cancer who had NGS of ctDNA. Median age at diagnosis was 50 years (range, 25 to 84 years). The majority of patients had metastasis or recurrence when blood was drawn for ctDNA testing (n = 90 [96%]) and had one or more nonsynonymous alterations (n = 74 [79%]; Table 1; Data Supplement).

ctDNA Sequencing Results

The median time from diagnosis to ctDNA analysis was 19.2 months (95% CI, 15.6 to 29.2 months). The median number of nonsynonymous alterations per patient was three (range, zero to 30). The most frequent nonsynonymous alterations were in *TP53* (52.1%), *KRAS* (34%), *APC* (28.7%), *BRAF* (19.1%), *PIK3CA* (17%), and *EGFR* (16%) genes (Fig 1A). Location of the primary colorectal cancer (right or left side) did not influence the frequency of these alterations.

Distinctiveness of genomic portfolios.—Among the 74 patients with one or more nonsynonymous ctDNA alterations, 59 (79.7%) had distinct gene alteration portfolios; 15 patients (20.3%) had similar genomic alteration portfolios (when alterations were considered at the gene level only, irrespective of the specific variant). This was the case only when patients had a small number of alterations (three or fewer) and included mostly the three genes with the most frequent alterations: *TP53*, *KRAS*, and *APC* (Fig 1B). Of note, all 15 patients had distinct genomic alterations when gene variants were considered. Thus, no two patients had identical molecular profiles (considering both genes and their loci). Among the 74 patients with one or more detectable non-synonymous ctDNA alterations, 93.2% (69 of 74 patients) had one or more characterized anomalies that were potentially actionable by FDA-approved drugs (82.4%) or by experimental drugs (10.8%) in a clinical trial if an FDA-approved drug was not available (Fig 1C-D).

Comparison of ctDNA and tissue NGS testing.—Overall, 76 patients (80.6%) had tissue NGS (Foundation Medicine; see Patients and Methods). The median time interval between the tissue biopsy and ctDNA testing was 5.8 months (range, 0.03 to 81 months; 95% CI, 3.2 to 8.3 months). We found statistically significant correlations between driver alterations detected in ctDNA (*TP53*, *KRAS*, *APC*, and *BRAF*) when compared with those detected in tissue, with concordance rates ranging from 63.2% to 85.5%, depending on the genes (Appendix Fig A1; Data Supplement). We did not observe a difference in the concordance rate when the time interval between the blood draw and tissue biopsy were taken into consideration (a cutoff of 6 months was used because it was the median time interval). Although *APC* alterations seemed to be detected more frequently in the tissue than in the plasma (22 patients were positive in both tests, 27 patients were positive in tissue only, and one patient was positive in plasma only), *BRAF* alterations seemed to be detected more frequently in the ctDNA test (eight patients were positive in both tests, two were positive in tissue only, and nine were positive in plasma only). Of note, there was 100% concordance between ctDNA and tissue DNA testing specifically for *BRAF*^{V600E} mutation (n = 6). In contrast, *BRAF* amplification was seen only with ctDNA testing (n = 8).

OS Analysis

First, we analyzed the impact of several clinical variables on OS calculated from the time of diagnosis (Table 2). In univariable analysis, the presence of nonsynonymous alterations in *APC*, *BRAF*, *EGFR*, *MYC*, and *ERBB2* genes from ctDNA and the presence of one or more gene alterations with ctDNA of 5% or more correlated with a poorer survival (all $P < .05$). Of interest, patients with metastases localized in their lungs had a statistically significantly improved survival in univariable analysis. After the multivariable analysis, patients in whom

an alteration was detected with ctDNA of 5% or more still had statistically significant survival (Fig 2A).

Among 76 patients who had tissue NGS analysis, patients with *BRAF* alterations had significantly worse OS, and patients with lung metastases had better OS (statistically significant after the multivariable analysis). *EGFR*, *MYC*, and *ERBB2* alterations in tissue DNA were not assessed because of the small number of patients harboring these alterations (fewer than 10 patients in each group; Data Supplement).

When survival was calculated from the time of blood draw (Table 2), the presence of nonsynonymous alterations in *APC*, *BRAF*, *PIK3CA*, *EGFR*, *MYC*, and *ERBB2* genes as well as alterations with ctDNA of 5% or more were associated with a shorter median OS (all $P < .05$). However, only *ERBB2* alterations remained significant in the multivariable model (Fig 2B).

Analysis of Outcomes for Patients With Matched Therapy Versus Patients With Unmatched Therapy

Outcomes of the patients who were treated with matched therapy after their ctDNA testing ($n = 17$) versus those who were given unmatched therapy ($n = 18$) were evaluated (Figure 3A; Data Supplement). Patients had a median of one prior therapy in the metastatic setting. Overall, we observed improved outcomes for patients with matched therapy (Fig 3). Altogether, 65% of patients in the matched therapy group attained SD for 6 months or more, PR, or CR versus 31% of patients in the unmatched therapy group ($P = .060$ in univariable analysis; $P = .045$ in multivariable analysis [multivariable analysis was performed using line of therapy as a confounding variable]). In addition, patients in the matched therapy group had a median PFS of 6.1 months compared with 2.3 months for patients in the unmatched therapy group ($P = .143$ for univariable analysis; $P = .079$ for multivariable analysis). Finally, patients who received matched therapy survived longer than unmatched therapy group, with a median OS (calculated from the treatment start date) not reached (at 11.1 months) compared to 9.4 months, respectively (albeit not statistically significant; Data Supplement).

Illustrative Case: Serial ctDNA Testing in a Patient Who Progressed While Receiving Anti-EGFR–Based Therapy

A 49-year-old-man with metastatic rectal adenocarcinoma was started on fourth-line therapy with irinotecan plus cetuximab. The patient's baseline ctDNA when therapy was initiated showed alterations including *APCE422** (2.2%) and *TP53 S127F* (1.9%) (Fig 4A). Tumor regression was initially seen, but upon progression that included new lung metastases and lymphangitic spread (Fig 4B), ctDNA among previously observed alterations increased approximately 20-fold (33.8% for *APCE422**; 39% for *TP53 S127F*), and emerging alterations included *APC I1307fs* and *EGFR* amplification (Fig 4A).

DISCUSSION

Despite the expansion of our recent understanding of the molecular biology of colorectal cancer^{6,7} and the development of salutary systemic therapies for advanced-stage disease,

overall prognosis remains poor.^{4,5} Thus, there is an urgent need for innovative treatment approaches for patients with metastatic colorectal cancer. Here we present the biologic features and clinical correlates of genomic alterations among 94 patients with mostly advanced-stage colorectal cancer by using targeted NGS that assessed ctDNA.

Overall, 79% of patients (74 of 94) had one or more nonsynonymous ctDNA alterations (Table 1). The most frequently characterized alterations were in *TP53* (51% of patients) followed by *KRAS* (34%), *APC* (27%), *BRAF* (16%), *PIK3CA* (16%), and *EGFR* (15%) genes (Fig 1). Although the frequency of *APC* alterations detected in this study is less than what has been previously reported, frequencies of other genomic alterations are consistent with those in previous publications.^{21,22} Concordance was statistically significant between ctDNA and tissue DNA among the driver or truncal gene alterations (overall concordance: *TP53*, 68%; *KRAS*, 74%; *APC*, 63%; *BRAF*, 86% [all $P < .05$]). Hong et al²⁹ previously reported that the ctDNA NGS had 100% sensitivity for tissue-detected as well as digital droplet polymerase chain reaction-based plasma-detected *BRAF*^{V600E} mutation among patients with colorectal cancer, which is consistent with our data. Considering that *BRAF*^{V600E} is targetable with a combination of anti-EGFR and anti-BRAF therapies, testing for this alteration is important.¹² It should be noted that some of the high overall concordance was driven by negative concordance. Sensitivity of ctDNA was variable for detection of mutations found in tissue. For instance, sensitivity of ctDNA for detection of patients with tissue *APC* positivity was 44.9%; sensitivity of ctDNA for detection of those with tissue *BRAF* positivity was 80% (this analysis was not restricted to *BRAF*^{V600E}). The apparent low capacity for detecting *APC* alterations could have implications for plasma monitoring of this alteration.

We did not observe differences in the concordance rate of driver alterations when we compared patients with a time interval between the blood draw and tissue biopsy of 6 months or less versus more than 6 months (Data Supplement). This observation differs from previous reports, which showed that the longer the time interval between the two tests, the lower the rate of concordance.^{20,30} The small number of patients in each of our subgroups may have confounded our ability to discern such differences.

Importantly, certain clinical and ctDNA factors were associated with survival outcome. When survival was evaluated from the time of blood draw to the last follow-up, the presence of *APC*, *BRAF*, *PIK3CA*, *EGFR*, *MYC*, and *ERBB2* nonsynonymous alterations (including single-nucleotide variations and amplifications) were all associated with poor overall survival (all $P < .05$ by univariable analysis), and *ERBB2* alterations remained significant after the multivariable analysis ($P < .001$) (Table 2; Fig 2). Many of these poor prognostic alterations are potentially targetable with either FDA-approved drugs (on- or off-label use) or with investigational agents currently in clinical development. Examples include using an EGFR inhibitor (eg, cetuximab) plus a BRAF inhibitor (eg, vemurafenib) for patients with *BRAF* alterations¹² and anti-HER2 agents (eg, trastuzumab and/or lapatinib) for *ERBB2* alterations.³¹

Accumulating evidence suggests that a biomarker-based approach to selecting targeted therapies, especially if they are based on genomic markers, is associated with improved

outcomes.^{32–36} Despite the relatively small numbers of patients in our study, we observed improvement in disease parameters in patients who received matched therapies (n = 17) compared with patients who received unmatched therapies (n = 18; SD was 6 months or more, PR, or CR [65% v 31%; $P = .045$]; median PFS, 6.1 v 2.3 months [$P = .079$]; median OS, not reached v 9.4 months [$P = .146$] using multivariable analysis; Figs 3–4; Data Supplement). The change in PFS and OS represented a nonsignificant trend, suggesting the need for larger studies. However, other factors may be operative and could have confounded our results. For instance, patients often received concomitant chemotherapy, and the putative interaction between *TP53* and bevacizumab could have inflated the results.^{37–39} These considerations are important because some matched therapies have not proved to be effective in colorectal cancers.^{40,41}

Although the precision medicine approach of matching patients with genomically or immunotargeted treatments is potentially promising, there are challenges for implementing this strategy (eg, tumor heterogeneity and genomic complexity among patients). In this study, we observed a median of three alterations per patient (range, zero to 30 alterations), and among 74 patients with one or more alterations, there were no two patients with identical molecular profiles. These findings suggest that matched monotherapy may not be optimal. Rather, customized combinations are most likely required for each individual. Preliminary results from the I-PREDICT (NCT02534675; Investigation of Profile-Related Evidence Determining Individualized Cancer Therapy) study of patients with aggressive malignancies showed a statistically significant improvement in time-to-treatment failure among patients receiving individualized combinations of matched therapies on the basis of genomic alterations when compared with patients receiving unmatched therapies.⁴² Expansion of this trial is ongoing.

We have also observed that patients who had high mutation allele frequency (MAF) and ctDNA of 5% or more had significantly worse survival (Table 2), which is consistent with previous reports.^{20,30} This observation is not surprising because ctDNA levels are reflective of the underlying tumor burden and can undergo dynamic changes after therapy.^{43,44} Moreover, Tie et al⁴⁵ reported that, among patients with early-stage colon cancer who had surgical resection, detection of postsurgical ctDNA was strongly associated with recurrent disease, which indicates that ctDNA can be a very sensitive biomarker for residual disease and treatment response. Along with this notion, we have also presented a patient with metastatic rectal adenocarcinoma whose ctDNA level increased by 20-fold when his tumor showed progression of lung metastases and lymphangitic spread while he was receiving treatment with irinotecan and cetuximab (Fig 4). It is unclear at this time whether increases in ctDNA level warrant a change in treatment strategy. Similarly, it is uncertain whether addition of new targeted therapy agents upon progression is warranted. In this study, *EGFR* amplification surfaced along with tumor progression while the patient was receiving anti-*EGFR* therapy (cetuximab; Fig 4). Emergence of alterations similar to those in ctDNA and other anomalies such as the appearance of *KRAS* alterations have been implicated in the evolution of resistance in patients with colorectal cancer who were treated with *EGFR* inhibitors.⁴⁶

There were several limitations to our study. First, it was performed retrospectively with a relatively small sample size at a single institution. Thus, our clinical findings require further validation with prospective studies. Second, multiple comparisons could result in overestimating the implications of *P* values; however, we have included a Bonferroni correction to attenuate this limitation. Third, it would be interesting to determine the impact of microsatellite stability status on the ctDNA alteration profile; this analysis could not be performed in this study because only 26 of our patients had microsatellite testing and 25 of them were microsatellite stable. Finally, because of the limited number of patients and the variable duration of time between tissue and ctDNA sampling, it was not feasible to analyze the effect of MAF for different alterations or the correlation between tissue and ctDNA MAF. Despite these limitations, our study provided an in-depth investigation of the clinical utility of ctDNA testing among patients with colorectal cancer.

In conclusion, we have interrogated tumors from 94 patients with mostly advanced-stage colorectal cancer who had clinical-grade NGS performed on blood-derived ctDNA. The median number of nonsynonymous alterations per patient was three and, importantly, each patient harbored a unique molecular profile. Concordance with common alterations in tissue ranged from 63% to 86%; differences between ctDNA and tissue could reflect dynamic changes in ctDNA after treatment. As was demonstrated by an illustrative patient, ctDNA may be shed from multiple metastatic sites or there could be differences in sensitivity between tissue and ctDNA sequencing. The presence of ctDNA of less than 5% independently correlated with longer survival, whereas *ERBB2* ctDNA nonsynonymous alterations were associated with shorter survival. Although the number of patients receiving targeted therapy in our study was modest, this is the first study, to our knowledge, to demonstrate objective evidence of the clinical utility of ctDNA NGS in metastatic colorectal cancer across multiple biomarkers beyond *BRAF*^{V600E}. Further clinical investigations using ctDNA to guide therapy in patients with colorectal cancer are needed.⁴⁷

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Appendix

Methods

Digital sequencing of circulating tumor DNA.

Circulating tumor DNA (ctDNA) sequencing was performed by Guardant Health, which was previously described (Guardant360, Redwood City, CA; <http://www.guardanthealth.com/guardant360/>). Guardant360 is a clinical laboratory certified by the Clinical Laboratory Improvement Amendment, accredited by the College of American Pathologists, and approved by the New York State Department of Health. The analytical and clinical validation of Guardant360 was conducted in conformance with evidentiary standards established by the Standards for Reporting of Diagnostic Accuracy, Reporting of Tumor Marker Studies, Evaluation of Genomic Applications in Practice and Prevention, recent next-generation sequencing (NGS), and Standardization of Clinical Testing Biomarker Guidelines.¹⁶

The sequencing uses molecular barcoding and hybrid capture followed by NGS of the critical exons in a panel of 54 to 73 genes and reports all four major types of genomic alterations (point mutations, insertions-deletions [indels], fusions, and copy number amplifications)^{16,23} See the Data Supplement for detailed gene panels. The analytic sensitivity reaches detection levels of one to two single mutant fragments from a 10-mL blood sample (0.1% limit of detection), and analytic specificity is greater than 99.9999%. The fractional concentration or mutation allele frequency (MAF) for a given somatic mutation is calculated as the fraction of ctDNA harboring that mutation in a background of wild-type ctDNA fragments at the same nucleotide position. The lower limit of detection of ctDNA was 0.04% for single nucleotide variants and fusions and 0.02% for indels.^{16,24} Only nonsynonymous alterations that include characterized alterations and variants of unknown significance (VUSs) were included in our analysis. When characterized alterations are referred to, VUSs were excluded in the analysis.

NGS of tissue.

Overall, 76 (81%) of 94 patients who had ctDNA results also had NGS performed by agencies accredited by Clinical Laboratory Improvement Amendment and the College of American Pathologists on tumor tissue using the FoundationOne assay (<http://www.foundationone.com/>; hybrid capture–based NGS; 182, 236, or 315 genes, depending on the time period). At > 250× median depth of coverage, > 99% of base substitutions present at MAF ≥ 10% were successfully detected. For indels at MAF ≥ 20%, 98% were detected. The methods have been previously described.²⁵

Concordance Rate

For the 76 patients who had both types of tests (tissue NGS and plasma ctDNA testing that covered the same genes and alteration types revealed in the tissue NGS), we assessed the concordance for the most frequent alterations and the corresponding kappa statistic, which is a conservative measurement of relative agreement that takes into account agreement by

chance. Kappa values range from 1 (perfect agreement) to 0 (no agreement other than what would be expected by chance).²⁶

Matched Therapy and Actionability

We retrospectively analyzed the treatments given after ctDNA testing and compared the clinical outcomes among patients who received matched and unmatched therapies. A therapy was considered matched if at least one agent in the treatment regimen targeted at least one abnormality or pathway component aberrant in a patient's ctDNA molecular profile. Patients were evaluable if therapy was administered for more than 10 days.

Actionability implies that the protein product of a genomic abnormality can be affected by a specific targeted drug.²⁷ A potentially actionable alteration was defined as an alteration that was either the direct target (such as an EGFR inhibitor targeting an *EGFR* mutation) or a pathway component (such as an mTOR inhibitor for a *PIK3CA* mutation [because mTOR is downstream of PIK3CA]) that could be targeted by at least one US Food and Drug (FDA)–approved or investigational drug in a clinical trial. Actionability was considered at the variant level; VUSs (functional consequences and clinical significance of these gene variants are not established, as opposed to characterized alterations) were considered nonactionable.

Statistical Analysis

Medians and 95% CIs or ranges were reported. Fisher's exact tests were used to compare categorical variables, and the nonparametric Mann-Whitney *U* test was used to compare two groups on one continuous variable. Binary logistic regressions were performed for categorical end points, and multiple linear regressions were performed for continuous variables. The rate of stable disease of 6 months or more, partial response, or complete response was compared between patients with matched and unmatched therapy. Stable disease, partial response, or complete response was determined per assessment of the treating physician. Progression-free survival was defined as the time from the beginning of therapy to progression or last follow-up date for patients who did not progress. Overall survival was defined as the time from diagnosis until death or last follow-up date for patients still alive. Progression-free survival and overall survival were analyzed by the Kaplan-Meier method,²⁸ and the log-rank test (univariable analysis) or Cox regression model (multivariable analysis) was used to compare variables. When appropriate, Bonferroni correction was used for multiple comparisons. Statistical analysis was performed by M.C.S. with SPSS version 24.0 (SPSS, Chicago, IL).

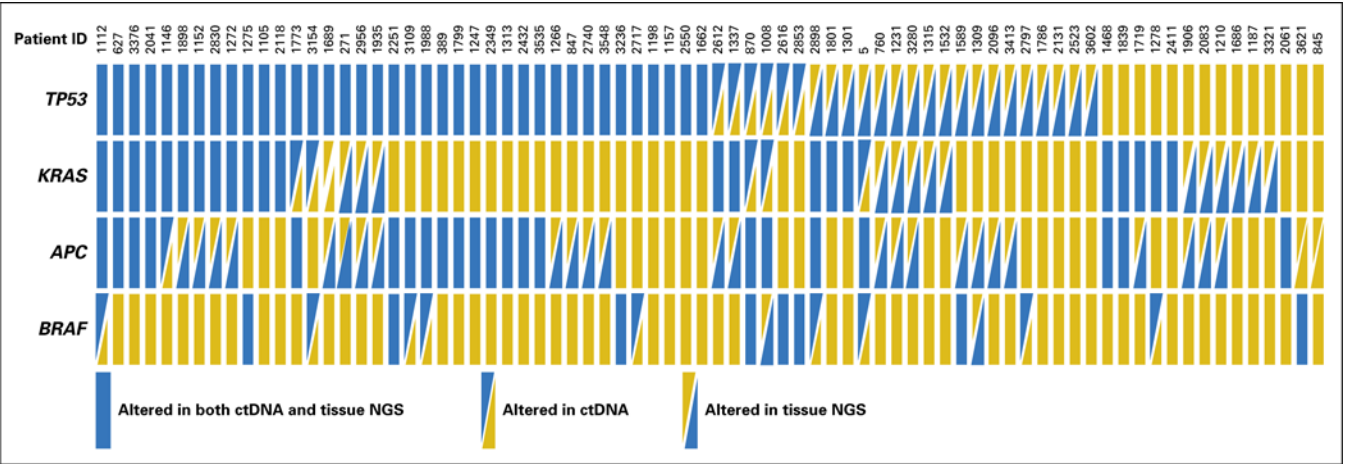


Fig A1.
Concordance between circulating tumor DNA (ctDNA) and tissue DNA analyses among commonly altered genomic alterations. NGS, next-generation sequencing.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

The following represents disclosure information provided by authors of this manuscript. All relationships are considered compensated. Relationships are self-held unless noted. I = Immediate Family Member, Inst = My Institution. Relationships may not relate to the subject matter of this manuscript. For more information about ASCO’s conflict of interest policy, please refer to www.asco.org/rwc or ascopubs.org/po/author-center.

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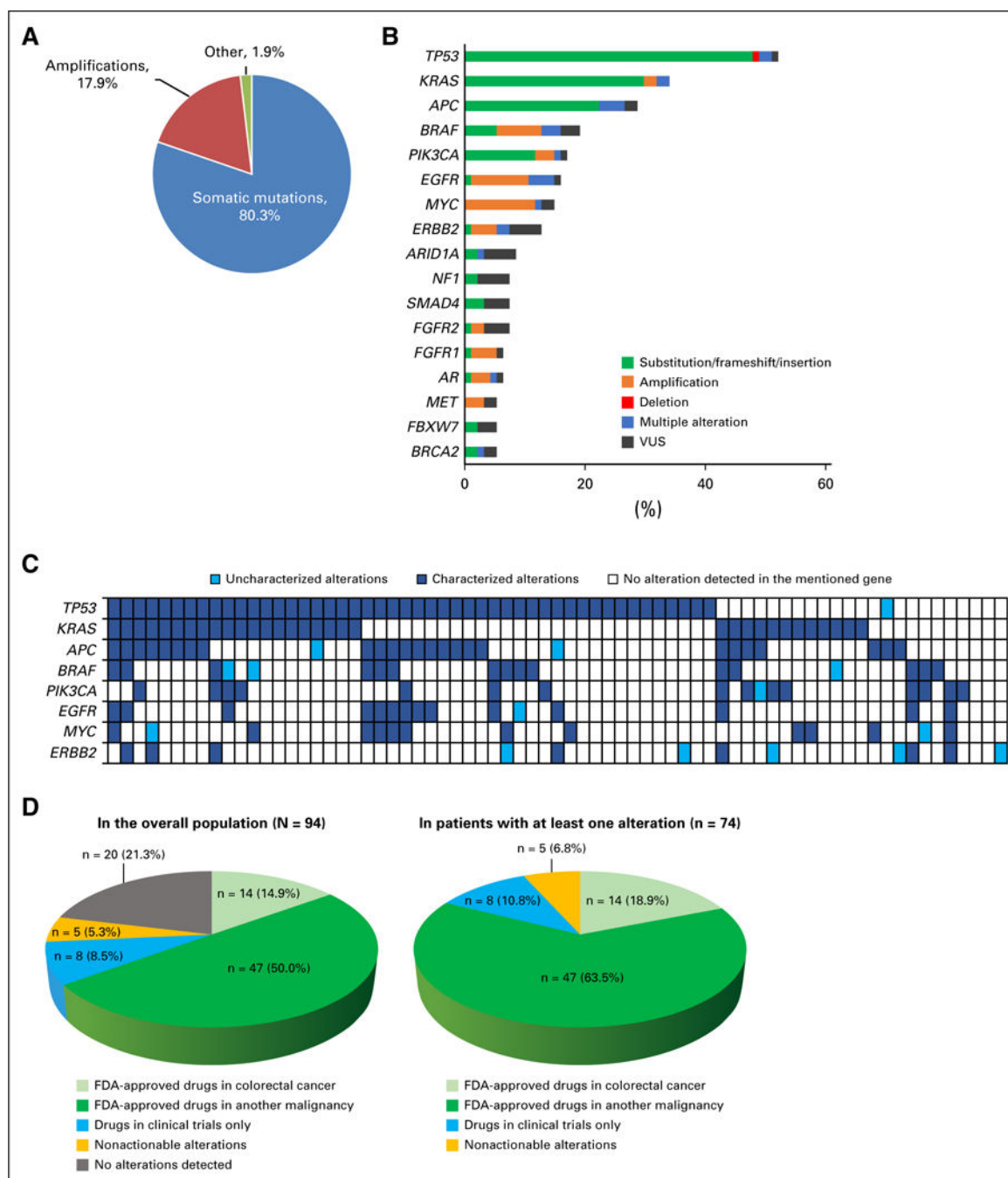
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**Fig 1.**

(A) Pie chart showing the types of alterations in the overall population (N = 94). In total, 375 nonsynonymous alterations were detected in 94 patients (74 patients had at least one alteration). Other alterations included three deletions, three insertions, and one fusion.. Frequencies are percent of alterations. (B) Frequency of the most common circulating tumor DNA (ctDNA) alterations. Only genes that were altered in five or more patients are displayed in the bar graph. The other altered genes in our population (fewer than five patients had the alteration) were *ABL1*, *AKT1*, *ALK*, *ARAF*, *ATM*, *BRCA1*, *CCND1*,

CCND2, CCNE1, CDH1, CDK6, CDKN2A, CTNNB1, ESR1, FGFR3, GNAQ, GNAS, HRAS, JAK3, KIT, MAP2K1, MLH1, MTOR, NOTCH1, NRAS, PTEN, RAF1, RB1, RET, RHOA, RIT1, ROS1, SDK6, SMO, STK11, VHL. This analysis included only nonsynonymous alterations. Alterations of unknown significance (variant of unknown significance; VUSs) versus characterized mutations (indels, amplifications, fusions, and single nucleotide variant (SNV) point mutations) were considered at the variant level. Multiple alteration indicates that different alterations were found in same gene (eg, alterations in both *BRAF* amplification and SNVs found in same patient). Frequencies are percent of patients. (C) OncoPrint of the most frequent alterations. Only alterations identified in more than 10 patients were represented. Each row represents the mentioned alteration; each column represents one patient. Only patients with at least one alteration in one of these genes—*TP53, KRAS, APC, BRAF, PIK3CA, EGFR, MYC, or ERBB2*—are displayed (n = 71 patients; the other 23 patients had no alterations in the represented genes and their corresponding columns would have been empty or white). (D) Pie charts representing the potential actionability of the detected alterations in the overall population (N = 94; left) and in patients with at least one nonsynonymous alteration (n = 74; right). Percentages are percent of patients. FDA, US Food and Drug Administration.

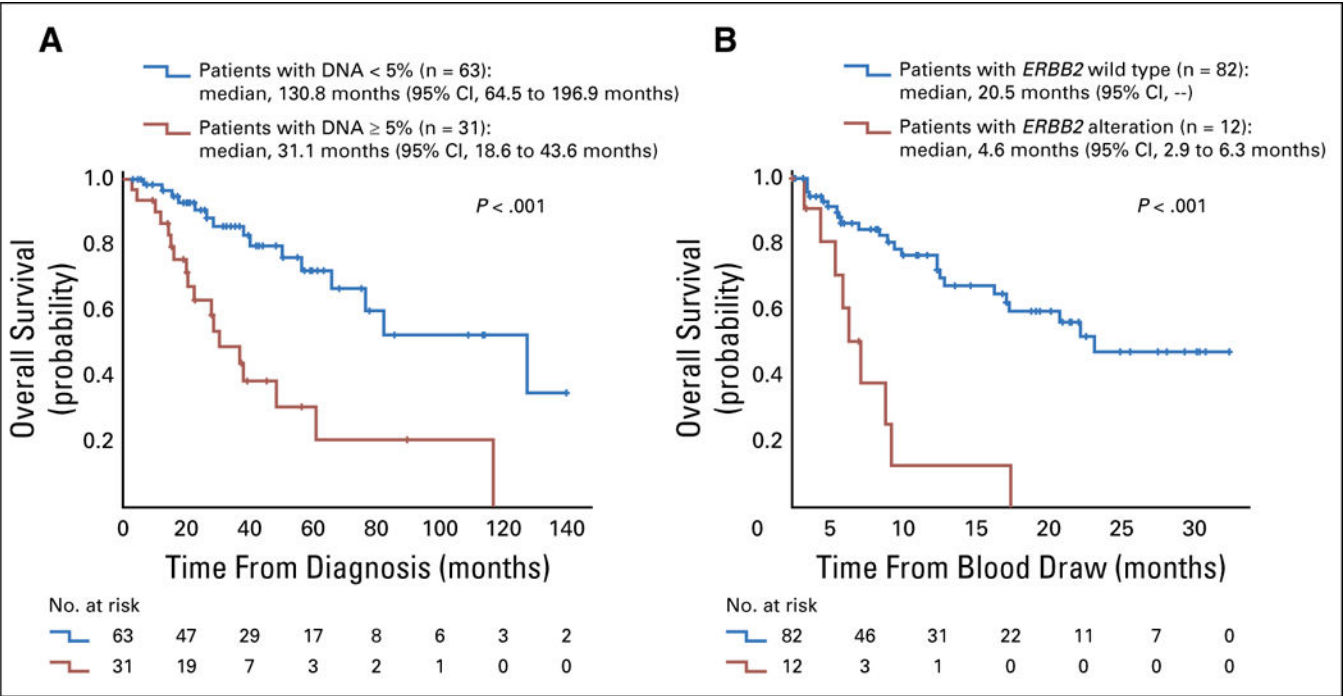
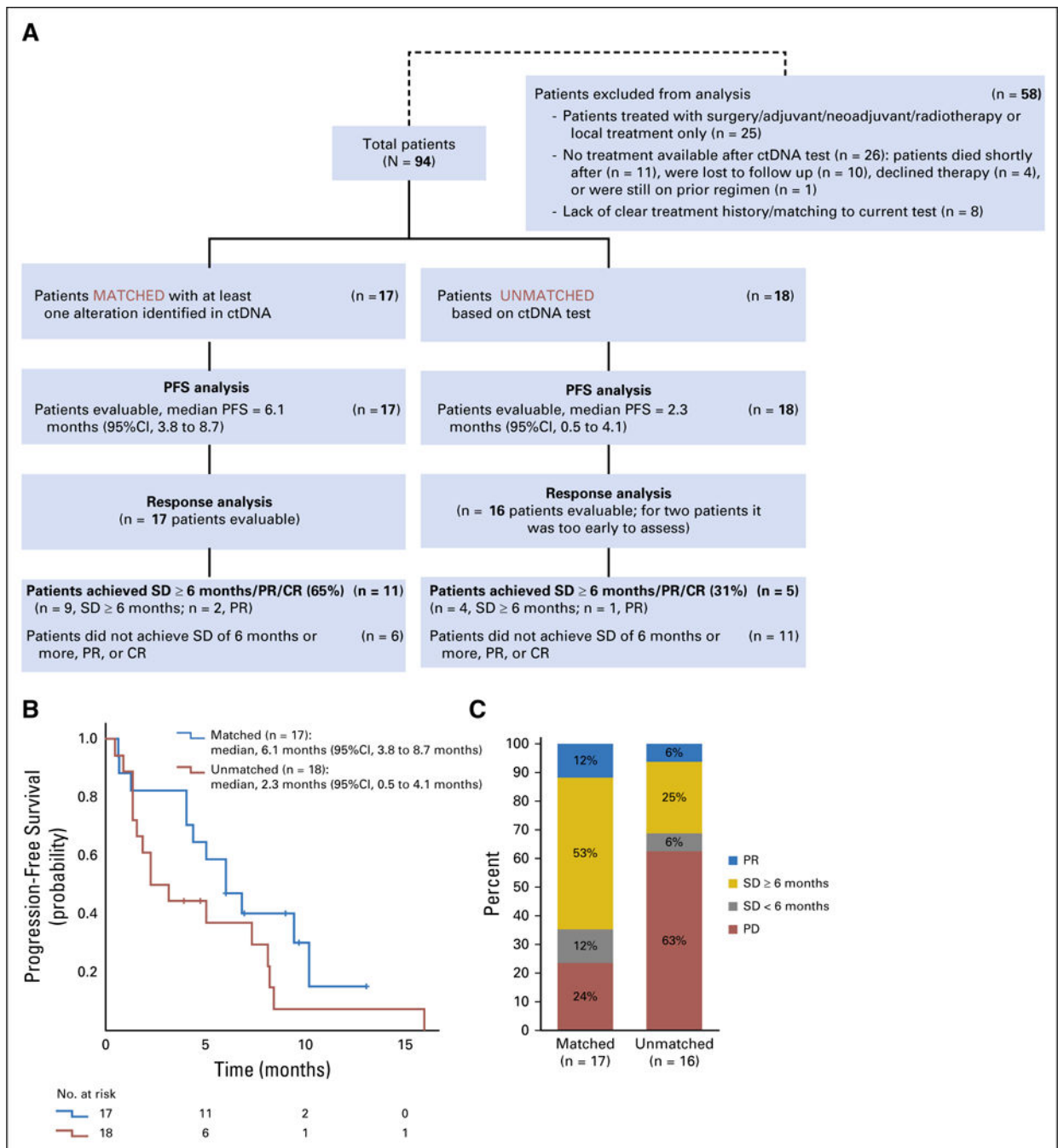


Fig 2. Kaplan-Meier curves for overall survival analysis from (A) the time of diagnosis and (B) the time of blood draw used for the ctDNA testing. The variables that were significant in the multivariable analyses (Table 2) are represented. *P* values are from the log-rank test.

**Fig 3.**

Treatment outcome analyses. (A) Diagram representing the treatment analyses and comparison of patients with matched treatment *v* patients with unmatched treatment. (B) Kaplan-Meier curves comparing the progression-free survival (PFS) for the patients with matched treatment (n = 17) *v* patients with unmatched treatment (n = 18). Univariable analysis (log-rank test) $P = .143$; multivariable analysis (Cox regression) $P = .079$. (C) Comparison of response outcomes for evaluable patients with matched treatment (n = 17) *v* those with unmatched treatment (n = 16). Univariable analysis (logistic regression) $P = .060$;

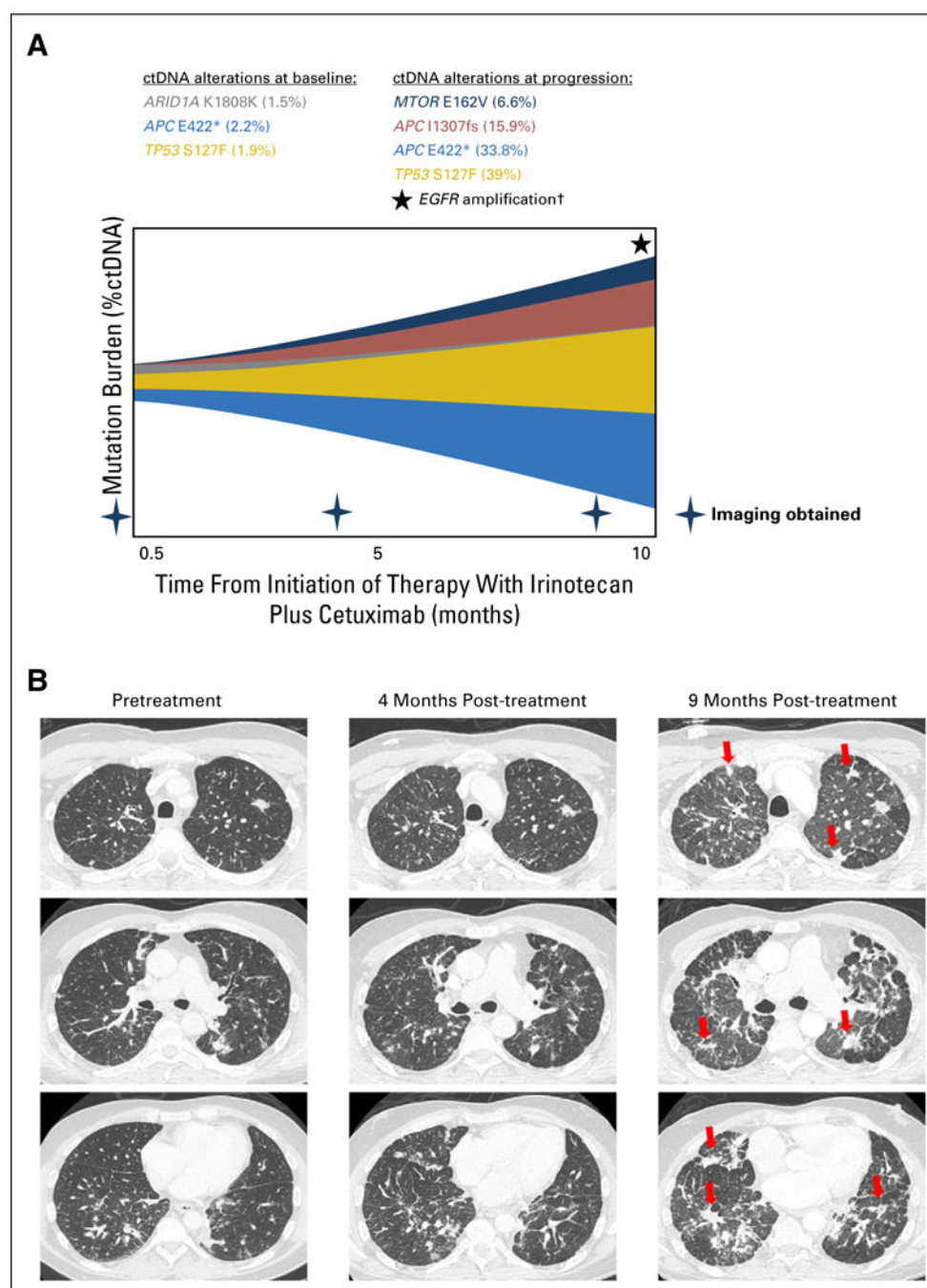
multivariable analysis (multiple logistic regression) $P = .045$. CR, complete response; ctDNA, circulating tumor DNA; PD, progressive disease; PR, partial response; SD, stable disease.

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**Fig 4.**

Patients who had serial circulating tumor DNA (ctDNA) testing and were progressing on anti-EGFR-based therapy. A 49-year-old-man with metastatic adenocarcinoma of the rectum had a history of previous treatment with (1) capecitabine plus oxaliplatin and (2) fluorouracil plus irinotecan plus bevacizumab; (3) treatment on a clinical trial with anti-CD73 included fourth-line therapy with irinotecan plus cetuximab. (A) Patient's baseline ctDNA at the start of therapy showing alterations (amount in percent). (B) The patient showed initial tumor regression, but at 9 months, the tumor progressed with new lung

metastases and lymphangitic spread (red arrow). ctDNA among previously observed alterations increased approximately 20-fold (33.8% for *APCE422**; 39% for *TP53 S127F*) along with emerging alterations, including *MTOR E162V*, *APC I1307fs*, and *EGFR* amplification. Among the ctDNA alterations observed in this patient, the following were characterized alterations: *TP53 S127F*, *APCE422**, *APC I1307fs*, and *EGFR* amplification. *MTOR E162V* was a variant of uncertain significance; *ARID1A K1808K* was a synonymous substitution. (†) Only levels of ctDNA mutations were quantified using %ctDNA and represented; *EGFR* amplification was detected at progression but not quantified.

Table 1.

Characteristics of Patients With Colorectal Cancer (N = 94)

| Characteristic | No. | % | Median | Range | 95% CI |
|---|-----|------|--------|-----------|--------------|
| Sex | | | | | |
| Female | 43 | 45.7 | | | |
| Male | 51 | 54.3 | | | |
| Ethnicity | | | | | |
| White | 61 | 64.9 | | | |
| Hispanic | 16 | 17 | | | |
| Other [*] | 17 | 18.1 | | | |
| Age at diagnosis, years | | | 50.1 | 24.9–83.9 | |
| Metastasis/recurrence at the time of blood draw | 90 | 95.7 | | | |
| Therapies in metastatic setting at time of ctDNA test | | | 1 | 0–5 | |
| Time from diagnosis to ctDNA results, months | | | 19.2 | | 15.6 to 29.2 |
| Location of colorectal cancer [†] | | | | | |
| Right | 49 | 52.1 | | | |
| Left | 45 | 47.9 | | | |
| Patients with one or more alterations [‡] | 74 | 78.7 | | | |
| Alterations per patient [‡] | | | 3 | 0–30 | 2 to 3 |
| Characterized alterations per patient ^{‡§} | | | 2 | 0–25 | 1.5 to 2 |

Abbreviation: ctDNA, circulating tumor DNA.

^{*} Included Asian (n = 4), African American (n = 3), and unknown (n = 10).

[†] Sigmoid and cecum were considered right colon. All other locations were considered left colon for the purpose of our analysis.

[‡] Included only nonsynonymous alterations.

[§] Variants of unknown significance were excluded.

Table 2.

OS Analyses (N = 94)

| Characteristic | Univariable Analysis | | Multivariable Analysis | |
|--|---------------------------------|---------------------------|------------------------|-------------------------|
| | Median (standard error), months | P | Wald Statistic | P |
| OS from diagnosis | | | | |
| Men (n = 51) v women | 130.8 (63.7) v 67.4 (12.0) | .966 | — | — |
| Location of colorectal cancer on the right (n = 49) v left | 57.8 (14.1) v 84.3 (15.8) | .306 | — | — |
| Genetic alteration type | | | | |
| <i>TP53</i> (n = 49) v wild type | 84.3 (23.9) v 62.3 (6.2) | .582 | — | — |
| <i>KRAS</i> (n = 32) v wild type | 78.5 (33.4) v 67.4 (11.7) | .478 | — | — |
| <i>APC</i> (n = 27) v wild type | 49.5 (8.3) v 78.5 (13.4) | .045 | — | — |
| <i>BRAF</i> (n = 18) v wild type * | 29.3 (7.4) v 84.3 (19.4) | .011 | — | — |
| <i>PIK3CA</i> (n = 16) v wild type | 41.0 (20.5) v 84.3 (19.0) | .057 | — | — |
| <i>EGFR</i> (n = 15) v wild type | 31.1 (5.1) v 84.3 (21.9) | .003 | — | — |
| <i>MYC</i> (n = 14) v wild type | 38.8 (10.3) v 84.3 (22.1) | .008 | — | — |
| <i>ERBB2</i> (n = 12) v wild type * | 37.7 (12.6) v 84.3 (21.9) | .001 | — | — |
| One or more alterations with ctDNA | 5% (n = 31) v not | 31.1 (6.4) v 130.8 (33.8) | <.001 | 16.4 <.001 [†] |
| Metastatic sites | | | | |
| Lung (n = 54) v not [‡] | 84.3 (24.2) v 67.4 (20.2) | .033 | 6.4 | .011 |
| Liver (n = 65) v not | 57.8 (8.3) v 84.3 (7.1) | .215 | — | — |
| Lymph node (n = 45) v not | 62.3 (10.5) v 84.3 (24.0) | .458 | — | — |
| Omentum (n = 32) v not | 84.3 (19.5) v 78.5 (24.8) | .973 | — | — |
| Ovary (n = 11) v not | 84.3 (18.1) v 67.4 (25.5) | .595 | — | — |
| OS from the blood draw used for ctDNA testing | | | | |
| Men (n = 51) v women | 14.8 (—) v 19.5 (4.3) | .737 | — | — |
| Colorectal cancer located on the right (n = 49) v the left | 14.5 (5.2) v 18.1 (—) | .407 | — | — |
| Genetic alteration type | | | | |
| <i>TP53</i> (n = 49) v wild type | 14.7 (—) v 19.5 (4.1) | .878 | — | — |

| Characteristic | Univariable Analysis | | Multivariable Analysis | |
|------------------------------------|---------------------------------|--------|------------------------|---------------------|
| | Median (standard error), months | P | Wald Statistic | P |
| <i>KRAS</i> (n = 32) v wild type | 10.3 (4.7) v 20.5 (—) | .111 | — | — |
| <i>APC</i> (n = 27) v wild type | 9.8 (5.0) v 20.5 (—) | .035 | — | — |
| <i>BRAF</i> (n = 18) v wild type | 3.8 (0.7) v 19.5 (3.7) | .005 | — | — |
| <i>PIK3CA</i> (n = 16) v wild type | 6.4 (2.5) v 20.5 (—) | .005 | — | — |
| <i>EGFR</i> (n = 15) v wild type | 3.4 (1.2) v 20.5 (—) | .001 | 6.3 | .012 |
| <i>MYC</i> (n = 14) v wild type | 6.7 (1.5) v 19.5 (3.8) | .034 | — | — |
| <i>ERBB2</i> (n = 12) v wild type | 4.6 (0.9) v 20.5 (—) | < .001 | 12.1 | < .001 [‡] |
| One or more alterations with ctDNA | 5% (n = 31) v not | .001 | — | — |
| Metastatic sites | | | | |
| Lung (n = 54) v not | 18.1 (3.6) v 19.5 (4.8) | .706 | — | — |
| Liver (n = 65) v not | 14.8 (4.7) v 20.5 (7.2) | .567 | — | — |
| Lymph node (n = 45) v not | 13.7 (2.7) v NR | .064 | — | — |
| Omentum (n = 32) v not | NR v 14.7 (2.3) | .526 | — | — |
| Ovary (n = 11) v not | 10.0 (0.3) v 18.1 (3.0) | .907 | — | — |

NOTE. This table includes only those variables with at least 10 patients having the designated characteristic. In some instances, the standard error could not be computed (—). Only variables that were significant in the univariable models (log-rank test) were included in the multivariable analysis (Cox regression model), with the final model containing only significant covariables in the multivariable analyses (forward stepwise selection model). All of the alterations listed are nonsynonymous (variants of unknown significance [VUSs] are included). The Wald statistic tests the unique contribution of each variable; the higher the Wald statistic, the higher the association or contribution in the model.

Abbreviations: ctDNA, circulating tumor DNA; NR, not reached.

*If only alterations are included in the multivariable analysis, *BRAF* ($P = .034$) and *ERBB2* ($P = .004$) remain significantly associated with shorter survival. For analysis of ctDNA of 5% or more, all nonsynonymous alterations were included. When examining the detailed results, all but one patient had one or more characterized alterations with ctDNA of 5% or more (in one patient, the alteration with ctDNA of 5% or more was classified as a VUS [ROS1 D1776H; 35.23%]).

[‡] Bonferroni-corrected P value level of significance was .007 (corrected for multiple comparisons). Only the variables with $P < .007$ were considered significant.

[§] In multivariable analysis, lung metastases correlated with longer survival.